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Applicant(s):	Marianne BRUGGEMANN	Confirmation No.:	3627
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Filing Date:	December 13, 2000	Group Art Unit:	1632
Title:	MURINE EXPRESSION OF A HUMAN IGA LAMBDA LOCUS		

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In support of this claim, filed herewith is a certified copy of said original foreign application:

GB9823930.4 filed November 3, 1998

Respectfully submitted,

September 27, 2004
Date

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Murine Expression of Human Igλ Locus

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INTRODUCTION

The light chain component of the Ig protein is encoded by two separate loci, Igκ and Igλ. The proportion of antibodies containing κ or λ light chains varies considerably between different species (1-3), eg in mice the κ:λ ratio is 95:5, compared to 60:40 in humans. Two models have evolved to account for this apparent bias in the expression of κ in the mouse. Firstly, from the observation that murine Igλ-producing myelomas have rearranged κ light chain genes, and that Igκ-producing cells have the λ light chain locus in germline configuration, it was proposed that κ rearrangement must occur before λ rearrangement can commence (4, 5). In the human situation, however, while almost all λ producing cells have both κ alleles rearranged, the proportion of κ and λ producing cells are similar (4). The second proposal is that κ and λ loci are both available for rearrangement at the same time, but the mouse κ locus is more efficient at engaging the rearrangement process (reviewed in 6). The occasional finding of cells with rearranged λ and the κ locus in germline configuration may support this (5, 7, 8). The influence of antigen selection on the biased κ:λ ratio is discounted by the finding that the ratio is similar in fetal liver and in cells that have not encountered antigen (9-13).

Light chain V-J rearrangement occurs at the transition from pre B-II to immature B cells, where the surrogate light chain associated with membrane Igμ is replaced by κ or λ light chain (14). Although the timing of light chain rearrangement is essentially defined, the processes which activate light chain locus rearrangement are not fully understood. From locus silencing experiments, it became clear that κ

rearrangement is not a prerequisite for λ recombination (15). Indeed, κ and λ rearrangements are independent events (16), the activation of which may be affected by differences in the strength of the respective enhancers. A region believed to be important in the regulation of the accessibility of the human λ locus has been identified about 10 Kb downstream of Cλ7 (17, 18). Functional comparisons in reporter gene assays identified a core enhancer region that is flanked by elements which can drastically reduce enhancer activity in pre-B cells (17). Although transfection studies showed that the κ and λ 3' enhancer regions appear to be functionally equivalent, other (functional) sequences flanking the core enhancer motifs are remarkably dissimilar. Targeted deletion of the κ 3' enhancer in transgenic mice showed that this region is not essential for κ locus rearrangement and expression but is required to establish the κ:λ ratio (19).

The human Igλ locus on chromosome 22q11.2 is 1.1 Mb in size and typically contains 70 Vλ genes and 7 Jλ-Cλ gene segments (20, 21 and ref. therein). About half of the Vλ genes are regarded as functional and Jλ-Cλ1, 2, 3 and 7 are active. The Vλ genes are organised in three clusters which contain distinct V gene family groups. There are 10 Vλ gene families, with the largest (VλIII) being represented by 23 members. In human peripheral blood lymphocytes, the most J-C proximal V gene segments in cluster A, from families I, II and III, are preferentially rearranged, with the contribution of the 2a2 Vλ segment (2-14 in the new nomenclature [22]) being unusually high (23). All λ gene segments have the same polarity which allows deletional rearrangement (24). Sequence diversity of the Igλ repertoire is

provided mainly by Vλ-Jλ combination. Additional CDR3 diversity due to N (non-encoded)- or P (palindromic)-nucleotide additions at the V to J junction, although not as extensive as seen in IgH rearrangement, seems to be much more frequently used in humans than in mice (25, 26, 27, 28), where the TdT (terminal deoxyribonucleotide transferase) activity is down-regulated at the time of light chain rearrangement.

Here we have introduced a 410 Kb YAC, which contains most of the Vλ genes of cluster A and all the Jλ-Cλ segments in germline configuration, into mice which have one or both endogenous Igκ alleles disrupted. The translocus shows high expression in both backgrounds, and is able to compete equally with the endogenous mouse κ locus.

SUMMARY OF THE INVENTION

Transgenic mice were created carrying a 380 Kb region of the human immunoglobulin (Ig) λ light (L) chain locus in germline configuration. The introduced translocus on a yeast artificial chromosome (YAC) accommodates the most proximal V (variable gene) λ cluster - with 15 Vλ genes that contribute to over 60% of λ light chains in man - and all Jλ-Cλ segments with the 3' region including the downstream enhancer. The HuIgλYAC mice were bred with animals in which mouse κ L chain production was silenced by gene targeting. Human Igλ expression in mouse $\kappa^{-/-}$ animals was dominant with up to 84% of $B220^{+}$ B-cells expressing surface human L chain. In serum human Igλ was up to 1.9 mg/ml, whilst mouse L chain levels were reduced to 0.2 mg/ml. However, a striking result was that in heterozygous $\kappa^{+/-}$ and normal $\kappa^{+/+}$ translocus mice both human λ and mouse κ were expressed at similar high levels (38% and 45% of cells, respectively). Interestingly, in HuIgλYAC/Moκ mice human λ is predominantly expressed at the pre B-cell stage with subsequent upregulation of cells expressing mouse L chain at the immature B-cell stage. The human Vλ genes hypermutate readily but show restricted P or N sequence variability at the V-J junction. The finding that human λ genes can be utilised with similar efficiency in mouse and man implies that L chain expression is critically dependent on the configuration of the locus. Thus, the transfer of large transloci may circumvent many expression problems encountered with small gene constructs introduced into cells and animals, with the advantage that some silencing approaches such as exploiting human antibody production may prove unnecessary.

Thus, according to the invention, transgenic mice have been produced in which the proportion of κ and λ light chains resembles that found in humans. Mice of the invention can be characterised by exhibiting relative proportions of ≥ 60% κ light chains and ≥ 40% λ light chains. Such a κ:λ ratio of 60:40 or less (i.e. even higher λ and lower κ levels) is remarkable in view of the normal mouse ratio of about 95:5.

Figure legends

Figure 1. The HulgλYAC accommodates a 380 Kb region of the human λ light chain locus in authentic configuration with all Vλ genes of cluster A (21, 40, 54), the Jλ-Cλ segments and the 3' enhancer (17). Black boxes represent functional Vλ genes (3-27, 3-25, 2-23, 3-22, 3-21, 3-19, 2-18, 3-16, 2-14, 2-11, 3-10, 3-9, 2-8, 4-3, 3-1) and white boxes show Vλ genes with open reading frames (2-33, 3-32, 3-12) which have not been identified in productive rearrangements of human lymphocytes (40). Pseudogenes are not shown. Black triangles indicate rearranged V genes found by RT-PCR in spleen and sorted Peyer's patch cells from Hulgλ mice. The unique NotI restriction site is indicated. Probes to assess the integrity of the HulgλYAC, LA (left arm) and Cλ2+3 are indicated.

Figure 2. Southern blot analysis of HulgλYAC Integration. (Left) NotI digested testis DNA resolved on PFGE and hybridized with the Cλ2+3 probe. The same size band was obtained with the left arm probe (not shown). The majority of the hybridization signal remains in the compression band (CB) presumably due to protection of the NotI site by methylation. (Right) EcoRI/HindIII digests hybridized with the Cλ2+3 probe. Lane 1: HulgλYAC ES cell DNA from a protoplast fusion clone; lane 2: normal ES cell DNA; lane 3: human genomic DNA (XZ); lane 4: human KB carcinoma (55) DNA; lane 5 and 6: tail DNA from 2 HulgλYAC germline transmission mice. Note that the human DNA shows an additional 5.2 Kb band which represents an allelic variation (56).

Figure 3. Human Igλ, mouse Igκ and mouse Igλ serum titers for HuλYAC/Moκ$^{+/-}$ and HuλYAC/Moκ$^{-/-}$ mice (5-6 mice per group kept in germfree conditions and 5 human sera). Antibody levels presented were obtained from 2-3 months old animals but the serum titers from older mice were similar. From the five HuλYAC/Moκ$^{+/-}$ mice tested 3 animals had somewhat higher mouse Igκ titers than human Igλ whilst 2 animals showed higher human Igλ levels. The controls show light chain distribution in human and normal mouse serum. Total Ig levels are in good agreement with the sum of individual titers (not shown).

Figure 4. Flow cytometric analysis of light chain expression in the developing B-cell. A) κ and λ light chain distribution of CD19^{+} human periphal lymphocytes and B220^{+} mouse spleen cells from HuλYAC/Moκ$^{+/-}$ and HuλYAC/Moκ$^{-/-}$ mice. B) Mouse Igκ and human Igλ light chain distribution in gated populations of CD19^{+}/c-kit^{+} and CD19^{+}/CD25^{+} bone marrow cells.

Figure 5. Human Vλ sequences from sorted B220^{+} and PNA^{+} Peyer's patches B-cells from Hulgλ^{+}YAC/κ$^{+/-}$ mice.

MATERIALS AND METHODS

The HulgλYAC, introduction into embryonic stem cells and derivation of transgenic mice. The 410 Kb HulgλYAC, accommodating a 380 Kb region (Vλ-JCλ) of the human λ light chain locus with V, J and C genes in germline configuration, was constructed as described (29). To allow selection two copies of the neomycin resistance gene (NEO^r) were site-specifically integrated into the ampicillin gene on the left (centromeric) YAC arm. YAC-containing yeast cells were fused with HM-1 embryonic stem (ES) cells, a kind gift from D. Melton, as described (30) and G418 resistant colonies were picked and analysed 2-3 weeks after protoplast fusion. ES cells containing a complete HulgλYAC copy, confirmed by Southern hybridization, were used for blastocyst injection to produce chimeric

animals (31). Breeding of chimeric animals with Balb/c mice resulted in germline transmission. Further breeding with $\kappa^{-/-}$ mice (32) established the lines for analysis.

Southern blot analysis. Either conventional DNA was obtained (33) or high molecular weight DNA was prepared in agarose blocks (34). For the preparation of testis DNA, tissues were homogenized and passed through 70µM nylon mesh. PFGE conditions to separate in the 50-900 Kb range were 1% agarose, 180V, 70s switch time and 30 hours running time at 3.5°C. Hybridization probes were Cλ2+3 and the left YAC arm probe (LA) comprising *LYS2* (29).

Hybridoma production and ELISA assay. Hybridomas were obtained from three months old HulgλYAC/$\kappa^{+/-}$ animals by fusion of splenocytes with NS0 myeloma cells (35). After fusion cells were plated on 96-well plates such as to obtain single clones. Human and mouse antibody production was determined in sandwich ELISA assays (36) on MaxiSorp plates (Nalge Nunc, Denmark). For the detection of human or mouse Igλ, coating reagents were a 1:500 dilution of anti-human λ light chain monoclonal antibody (mAB) HP-6054 (L 6522, Sigma, St.Louis, MO, USA) or a 1:500 dilution of the 2.3 mg/ml rat anti-mouse λ mAB (L 2280, Sigma), respectively. Respective binding was detected with biotinylated antibodies: polyclonal anti-human λ (B 0900, Sigma), a 1:1000 dilution of polyclonal anti-mouse λ (RPN 1178, Amersham Intl., Amersham, UK) or rat anti-mouse Igλ (# 021172D, Pharmingen, San Diego, USA) followed by streptavidin-conjugated horseradish peroxidase (Amersham). Mouse IgG2aλ myeloma protein from HOPC1 (M 6034, Sigma) and human serum IgGλ (I 4014, Sigma) were used

to standardise the assays. To determine mouse κ light chain levels, plates were coated with a 1:1000 dilution of rat anti-mouse κ, clone EM34.1 (K 2132, Sigma), and bound Ig was detected using biotinylated rat mAB anti-mouse Igκ (Cat. no. 04-6640, Zymed, San Francisco, USA). Mouse myeloma proteins IgG2aκ and IgG1κ (UPC10, M 9144, and MOPC21, M 9269, Sigma) were used as standards. For detection of mouse IgM, plates were coated with polyclonal anti-mouse μ (The Binding Site, Birmingham, UK) and bound Ig was detected with biotinylated goat anti-mouse μ (RPN1176, Amersham) followed by streptavidin-conjugated horseradish peroxidase. Mouse plasmacytoma TEPC183, IgMκ, (M 3795, Sigma) was used as a standard.

Flow cytometry analysis. Cell suspensions were obtained from bone marrow (BM), spleen and Peyer's patches (PPs). Multicolour staining was then carried out with the following reagents in combinations illustrated in figure 4: FITC-conjugated anti-human λ (F5266, Sigma), PE-conjugated anti-mouse c-kit (CD117) receptor (clone ACK45, cat. no. 09995B, Pharmingen, San Diego, USA), PE-conjugated anti-mouse CD25 (IL-2 receptor) (clone 3C7, P 3317, Sigma), biotin-conjugated anti-human κ (clone G20-193, cat. no. 08172D, Pharmingen), biotin-conjugated anti-mouse CD19 (clone 1D3, cat. no. 09654D, Pharmingen), followed by Streptavidin-Quantum Red (S2899, Sigma) or Streptavidin-PerCP (cat. no. 340130, Becton-Dickinson) and rat monoclonal anti-mouse κ light chain (clone MRC-OX-20, cat. MCA152, Serotec, Oxford, UK) coupled according to the manufacturer's recommendations with allophycocyanin (APC) (PJ25C, ProZyme, San Leandro, USA). Data were collected from $1x10^6$ stained cells on a FACScalibur flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA)

as described (32). Cells were first gated on forward and side scatter to exclude dead cells. To obtain accurate percentage distribution for comparison, cells from normal mice were stained in parallel. In addition, human peripheral blood lymphocytes were purified on Ficoll gradients (1.077g/ml) and stained with PE-conjugated anti-human CD19 antibody (P7437, clone SJ25-C1, Sigma), biotinylated anti-human κ followed by Streptavidin-Quantum Red and FITC-conjugated anti-human λ antibodies as above.

For RT-PCR cloning of Vλ genes, PPs cells were stained with FITC-conjugated peanut agglutinin (PNA) (L 7381, Sigma) and PE-conjugated anti-mouse B220 antibodies (P 3567, Sigma). Double-positive cells were sorted on the FACStar^{Plus} flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA) as described (32) and $5x10^3$ cells were lysed in denaturing solution (37). 5'RACE was carried out as described below with one modification - 2μg carrier RNA was added to the cell lysates before RNA extraction and precipitation.

Cloning and sequencing of 5'RACE products. Spleen RNA was prepared as described (37) and for cDNA preparation 2-3 μg of RNA was ethanol precipitated and air-dryed. For rapid amplification of 5' cDNA ends (5'RACE) (38) first strand cDNA was primed with oligo(dT)22 and 100 units of Super Script II reverse transcriptase (Gibco BRL, Gaithersburg, MD, USA) was used at 46°C according to manufacturer's instructions with 20 units of placental RNAse inhibitor (Promega, Madison, WI, USA). The DNA/RNA duplex was passed through 1 ml G-50 equilibrated with TE (10mM Tris-HCl pH7.8, 1mM EDTA) in a hypodermic syringe to remove excess oligo(dT). For G-tailing 20 units of TdT (Cambio, Cambridge, UK) were used according to standard protocols (39). Double stranded (ds) cDNA was

obtained from G-tailed ss cDNA by addition of oligonucleotide Pr1 (see below), 100 µM dNTP and 2.5 units of Klenow fragment (Cambio) and incubation for 10 min at 40°C. After heating the reaction for 1 min at 94°C and extraction with phenol-chloroform the ds cDNA was passed through G-50 to remove primer Pr1. PCR amplifications, 35 cycles, were carried out in the RoboCycler Gradient 96 Thermal Cycler (Stratagene, LaJolla, CA, USA) using oligonucleotides Pr2 and Pr3. For PCR of PPs cDNA 50 cycles were used: 40 cycles in the first amplification and 10 cycles in additional amplifications. Pfu Thermostable Polymerase (Stratagene, LaJolla, CA, USA) was used instead of Taq polymerase to reduce PCR error rates. The amplification products were purified using a GENECLEAN II kit (BIO 101, Vista, CA, USA) and re-amplified for 5 cycles with primers Pr2 and Pr4 to allow cloning into Eco RI sites. Oligonucleotide for 5'RACE of Vλ genes were: Pr1 5'-AATTCTAAAACTACAAACTG CCCCCCCCCA/T/G-3', Pr2 5'-AATTCTAAAACTACAAACTGC-3' (sense), Pr3 -5'-CTCCCGGGTAGAAGTCAC-3' (reverse), Pr4 5'-AATTCGTGTGGCCTTGTTGGCT-3' (reverse nested).

The protocol of A. Sudarikov (pers.comm.) was used to clone Vλ PCR products. PCR products of about 500bp were cut out from agarose gels and purified on GENECLEAN II. The DNA was incubated in 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, with 100 µM dGTP/dCTP and 1 unit of Klenow fragment for 10 min at RT. Under these conditions the Klenow fragment removes the 3' ends of the PCR products (AATT) leaving ligatable Eco RI overhangs. DNA was ligated with Eco RI restricted pUC19, transformed into competent *E.coli* XL1Blue and colonies were selected on X-Gal/IPTG/amp plates. Plasmid DNA prepared from white colonies was used for sequencing. Sequencing of both strands was done on the ABI 373 automated sequencer in the Babraham Institute Microchemical Facility.

RESULTS

The transgenic human Igλ locus. The human Igλ translocus (Fig. 1) was assembled on a YAC by recombining one YAC containing about half of all Vλ gene segments with three overlapping cosmids containing Vλ and Jλ-Cλ gene segments and the 3' enhancer (29). This resulted in a 410 Kb YAC accommodating a 380 Kb region of the human λ light chain locus containing 15 Vλ genes regarded as functional, 3 Vλs with open reading frames not found to be expressed and 13 Vλ pseudogenes (40). This HulgλYAC was introduced into ES cells by protoplast fusion (30) and chimeric mice were produced by blastocyst injection (31). The ES cell clone used for this showed a 450 Kb NotI fragment corresponding to HulgλYAC, as identified by PFGE and Southern hybridization with the 3' probe, Cλ2+3, and the 5' probe, LA comprising *LYS2*, present in the left centromeric YAC arm (not shown). Germline transmission was obtained, and PFGE analysis of testis DNA from one animal is illustrated in fig. 2. A NotI fragment larger than 380 Kb is necessary to accommodate this region of the HulgλYAC, and the 450 Kb band obtained indicates random integration involving the single NotI site 3' of Jλ-Cλ and a NotI site in the mouse chromosome. Digests with EcoRI/HindIII and hybridization with the Cλ2+3 probe further confirmed the integrity of the transferred HulgλYAC (Fig. 2). The results indicated that one complete copy of the HulgλYAC was integrated in the mouse genome.

Human Igλ expression is dominant in mouse $\kappa^{-/-}$ animals. To assess the human λ light chain repertoire for the production of authentic human antibodies the HulgλYAC mice were bred with mice in which endogenous Igκ production was

silenced by gene targeting (32). In these $\kappa^{-/-}$ mice, the mouse Igλ titers are elevated compared to $\kappa^{+/+}$ strains (32, 41). Serum titrations (Fig. 3) showed that human Igλ antibody titers in HuIgλYAC/$\kappa^{-/-}$ mice are between 1 and 2 mg/ml which is up to 10 fold higher than average mouse Igλ levels. Interestingly, the mouse Igλ levels remained low in the HuIgλYAC/$\kappa^{-/-}$ mice, similar to the levels found in normal mice. High levels of human Igλ^{+} cells were also identified in flow cytometric analysis of splenic B-cells from HuIgλYAC/$\kappa^{-/-}$ mice (Fig. 4A) with human λ expressed on the surface of >80% of the B-cells whilst the number of mouse Igλ^{+} cells was always below 5% (data not shown).

Human Igλ expression equals mouse Igκ production. Assessment of human Igλ production in heterozygous HuIgλYAC^{+}/$\kappa^{+/-}$ mice allowed a detailed comparison of expression and activation of endogenous versus transgenic light chain loci present at equal functional numbers. Serum analysis (Fig. 3) of mice capable of expressing both human λ and mouse κ showed similar titers for human and mouse light chains. Human Igλ levels in HuIgλYAC/$\kappa^{+/+}$ transgenic mice were very similar to those in HuIgλYAC/$\kappa^{+/-}$ mice. Total Ig levels in HuIgλYAC^{+}/$\kappa^{+/-}$ mice were 1-2 mg/ml, with a typical contribution of about 51% mouse Igκ, 43% human Igλ and 6% mouse Igλ. However, a comparison of endogenous κ and human λ expression in individual sera from HuIgλYAC mice, and similarly from human volunteers, showed that λ/κ ratios can vary. For example, three of the

HuIgλYAC/$\kappa^{+/-}$ mice produced somewhat higher κ levels whilst in two mice the human λ levels were higher than the Igκ titers. In HuIgλYAC/$\kappa^{+/-}$ mice, similar high translocus expression was also found in $B220^+$ B-cells from different tissues, for example 38% of spleen cells expressed human λ and 45% mouse κ (Fig. 4A). These values resemble very much the levels in human volunteers as illustrated in Fig. 4A with 34% $Ig\lambda^+$ versus 51% $Ig\kappa^+$ in $CD19^+$ peripheral blood lymphocytes.

To assess whether the high contribution of the human λ translocus to the mature B cell repertoire is the result of selection at the mature B-cell stage, or alternatively from early translocus rearrangement, we examined light chain expression in bone marrow precursor B-cells. For this, the early B-cell markers c-*kit* and CD25 were used in four-colour stainings in combination with the B-cell lineage marker CD19 and human λ and mouse κ specific antibodies. Fig. 4B shows that human λ expression in HuIgλYAC/$\kappa^{+/-}$ mice occurs at an earlier stage of development than mouse κ light chain expression. Human λ expression can be detected at the unusually early $CD19^+$/c-*kit*$^+$pre B-I stage and is maintained in $CD19^+/CD25^+$ pre B-II cells. However, at the later immature to mature B-cell stage ($CD19^+$/c-*kit*$^-$/$CD25^-$) the proportion of mouse $Ig\kappa^+$ cells is significantly increased. This suggests that human λ light chains can rearrange at an earlier stage than mouse Igκ but that upregulation at the mature B-cell stage balances any disadvantages in the timing of rearrangement.

DNA rearrangement and diversification of a highly active human λ translocus. In order to assess whether the translocus expression levels were a direct result of early rearrangement capacity we analysed individual hybridoma clones. Results from 2 fusions suggest that human λ and mouse κ light chain producing cells were present in the spleen of HulgλYAC/$\kappa^{-/+}$ mice at similar frequencies. Furthermore, the antibody expression rates of human λ (2-20 µg/ml) or mouse κ (4-25 µg/ml) producing hybridomas were similar. In order to assess if human Igλ rearrangement must precede mouse Igκ rearrangement or *vice versa*, endogenous and transgene rearrangements were analysed. Southern blot hybridization of randomly picked human Igλ or mouse Igκ expressing hybridoma clones showed the following: of 11 human Igλ expressers, 7 had the mouse κ locus in germline configuration, 1 clone had mouse Igκ rearranged and 3 clones had the mouse κ locus deleted; and of 11 mouse Igκ expressers, all had the human Igλ locus in germline configuration. The analysis of 8 more Igκ producers showed that in 2 the human Igλ locus was rearranged (data not shown). This result suggests that there is no locus activation bias and further emphasises that the human λ translocus performs with similar efficiency as the endogenous κ locus.

The capacity of the human λ locus to produce an antibody repertoire is further documented in the V gene usage. V-J rearrangement was determined from spleen cells and Peyer's patch cells by PCR amplification without introducing bias from specific V gene primers. The results shown in Fig. 1 illustrate that a substantial proportion of the Vλ genes on the translocus are being used, with Vλ3-1 and Vλ3-

10 being most frequently expressed. In DNA rearrangement, Jλ2 and Jλ3 were preferentially used and Jλ1 rarely, and as expected Jλ4, 5 and 6 were not utilised as they are adjacent to ψCs. Sequences obtained by RT-PCR from FACS-sorted germinal centre $PNA^+/B220^+$ Peyer's patches (Figure 5) revealed that somatic hypermutation is operative in HulgλYAC mice (with somewhat more extensive changes in CDRs than in framework regions). Extensive variability due to N- or P-sequence additions, which is found in human but not mouse light chain sequences (25, 27, 28), was not observed.

DISCUSSION

Efficient DNA rearrangement and high antibody expression levels are rarely achieved in transgenic mice carrying immunoglobulin regions in germline configuration on minigene constructs. Competition with the endogenous locus can be eliminated in Ig knock-out strains, where transgene expression is usually good (42). Poor transloci expression levels could be a result of the failure of human sequences in the mouse background, or alternatively the lack of locus specific control regions which are more likely to be included on larger transgenic regions (43, 44, 45). The latter is supported by our finding that HulgλYAC mice express human Igλ and mouse Igκ at similar levels. The 410 Kb HulgλYAC translocus accommodates V-gene region cluster A containing at least 15 functional Vλ genes (see Fig. 1). In man, cluster A is the main contributor to the λ antibody repertoire, with Vλ 2-14 (2a2) expressed most frequently at 27% in blood lymphocytes (23). We also find expression of Vλ 2-14 in the transgenic mice but the main contributors

to λ light chain usage were 3-1, the Vλ gene most proximal to the C-J region, and 3-10, both of which are expressed at about 3% in man. Although the validity to draw conclusions about gene contribution is dependent on the numbers compared, from the31sequences obtained 11 were Vλ3-1 and 8 were Vλ3-10 which suggests that rearrangement or selection preferences are different in mouse and man. Sequence analysis revealed that there was very little further diversification by insertion of N or P nucleotides. In contrast, we found extensive somatic hypermutation of many rearranged human Igλ sequences, indicating that they are able to participate in normal immune responses. Indeed mutation levels in $B220^+/PNA^+$ PPs from Hulgλ YAC translocus mice were similar to what has been reported for mouse light chains (46). In the mouse, unlike in humans, untemplated light chain diversification is essentially absent and it was believed that this is because deoxynucleotidyl transferase is no longer expressed at the stage of light chain rearrangement (28, 47). This concept has been challenged by the discovery that mouse light chain rearrangement can occur at the same time as V_H to DJ_H rearrangements (48). Indeed our results also show light chain rearrangement at the pre B-I stage, with a substantial percentage of $CD19^+$ cells expressing human λ (see Fig. 4). Although the human λ translocus appears to be earlier activated than the κ locus in the mouse, rearranged human λ light chains did not accumulated much N region diversity as found in human periphal B-cells (27).

In the different species, the ratio of λ and κ light chain expression varies considerably (1-3, 49, 50) and in the mouse the low λ light chain levels are believed to be a result of inefficient activation of the mouse λ locus during B-cell

differentiation (reviewed in 6). The Igλ (~40%) and Igκ (~60%) ratio in humans is more balanced and suggests that both λ and κ play an equally important role in immune responses. This notion is supported by the finding that the mouse Vλ genes are most similar to the less frequently used distal human Vλ gene families, while no genes comparable to the major contributors to the human Vλ repertoire are present in mice (40). With the Hulgλ YAC, these Vλ genes are available, and are able to make a significant contribution to the antibody repertoire, and the bias towards Vκ gene utilisation is removed.

Comparison of size and complexity of light chain loci between different species suggests that larger loci with many more V genes may contribute much more efficiently to the antibody repertoire (6, 51). Recently we addressed this question in transgenic mice by the introduction of different size human κ light chain loci (45). The result showed that the size of the V gene cluster and the V gene numbers present are not relevant to achieving high translocus expression levels. It is possible, however, that a presently undefined region with cis-controlled regulatory sequences may be crucial in determining expressibility and subsequently light chain choice. That the HulgλYAC^{+}/κ$^{+/-}$ mice do not exhibit a bias in the selection of light chain locus for expression is shown by the absence of rearrangement of the non-expressed locus in hybridoma cells. This supports the model that λ and κ rearrangements are indeed independent (52) and that poor Igλ expression levels in mice may be the result of an inefficient recombination signal (53). A possible signal that initiates light chain recombination has been identified in gene targeting experiments where the 3' κ enhancer has been deleted (19). The κ:λ ratio was

essentially equal in mice where the 3'Eκ had been deleted or replaced by neo (down to 1:1 and not 20:1 as in normal mice). In addition, the κ locus was largely in germline configuration in λ expressing cells, a result we also see in the HuIgλYAC^{+}/κ$^{+/-}$ mice. Taken together, the results suggest that strength and ability of the human 3' λ enhancer to function in the mouse background may be the reason that human λ and mouse κ levels are similar in HuIgλYAC^{+}/κ$^{+/-}$ mice and that λ and κ light chain 3' enhancers compete at the pre B-cell stage to initiate light chain rearrangement.

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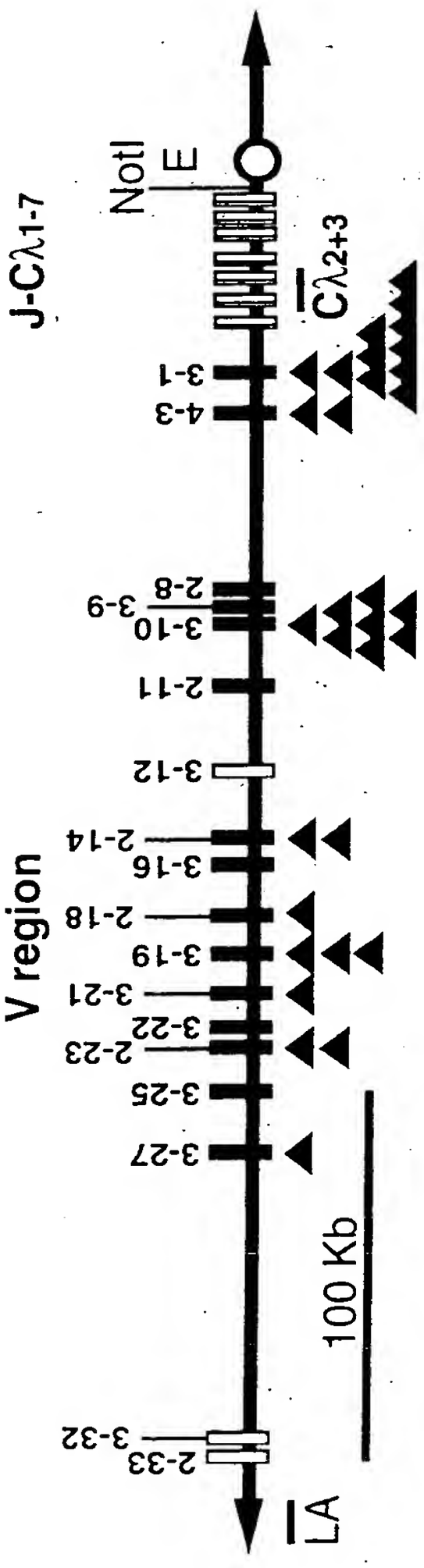
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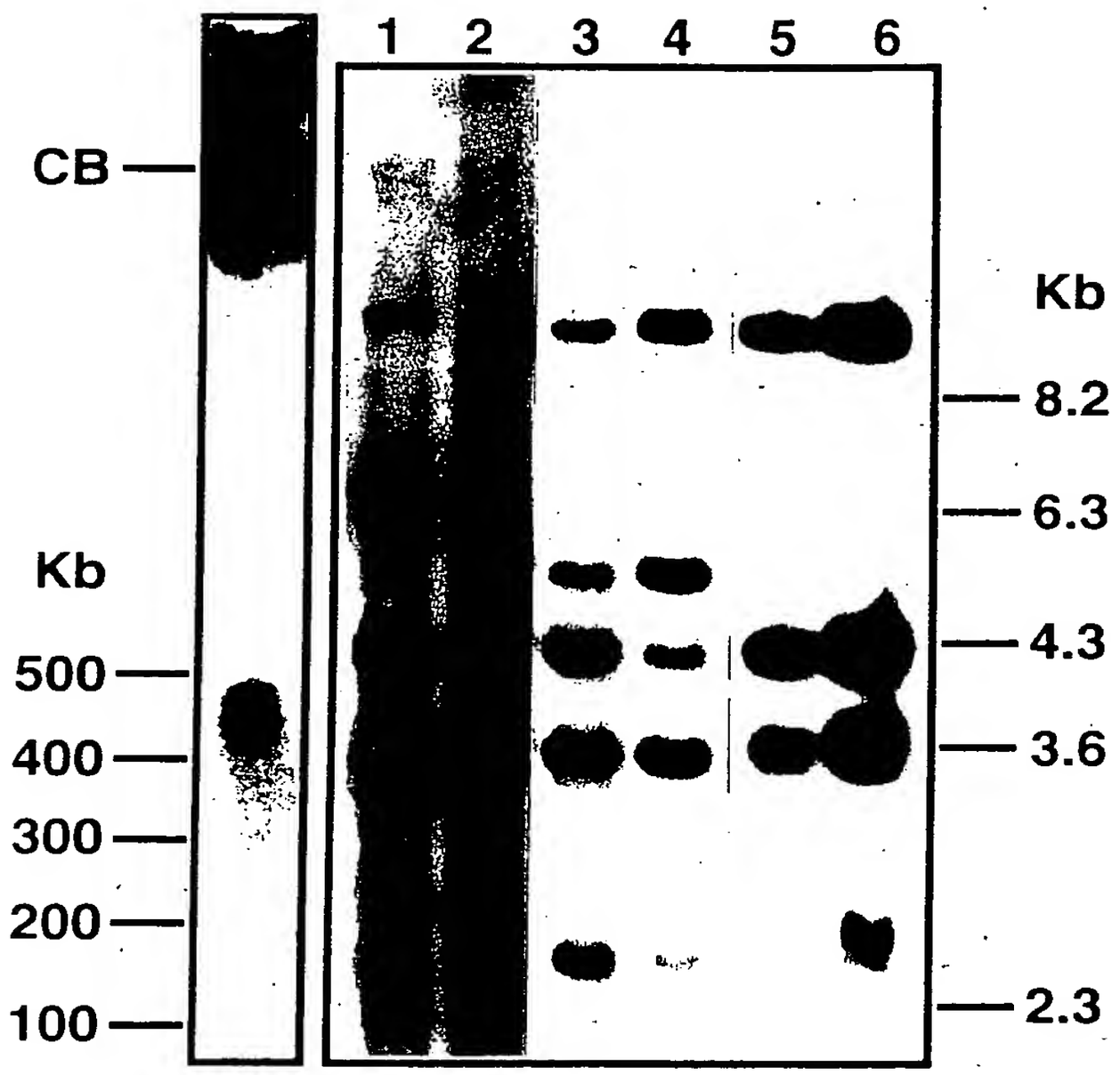
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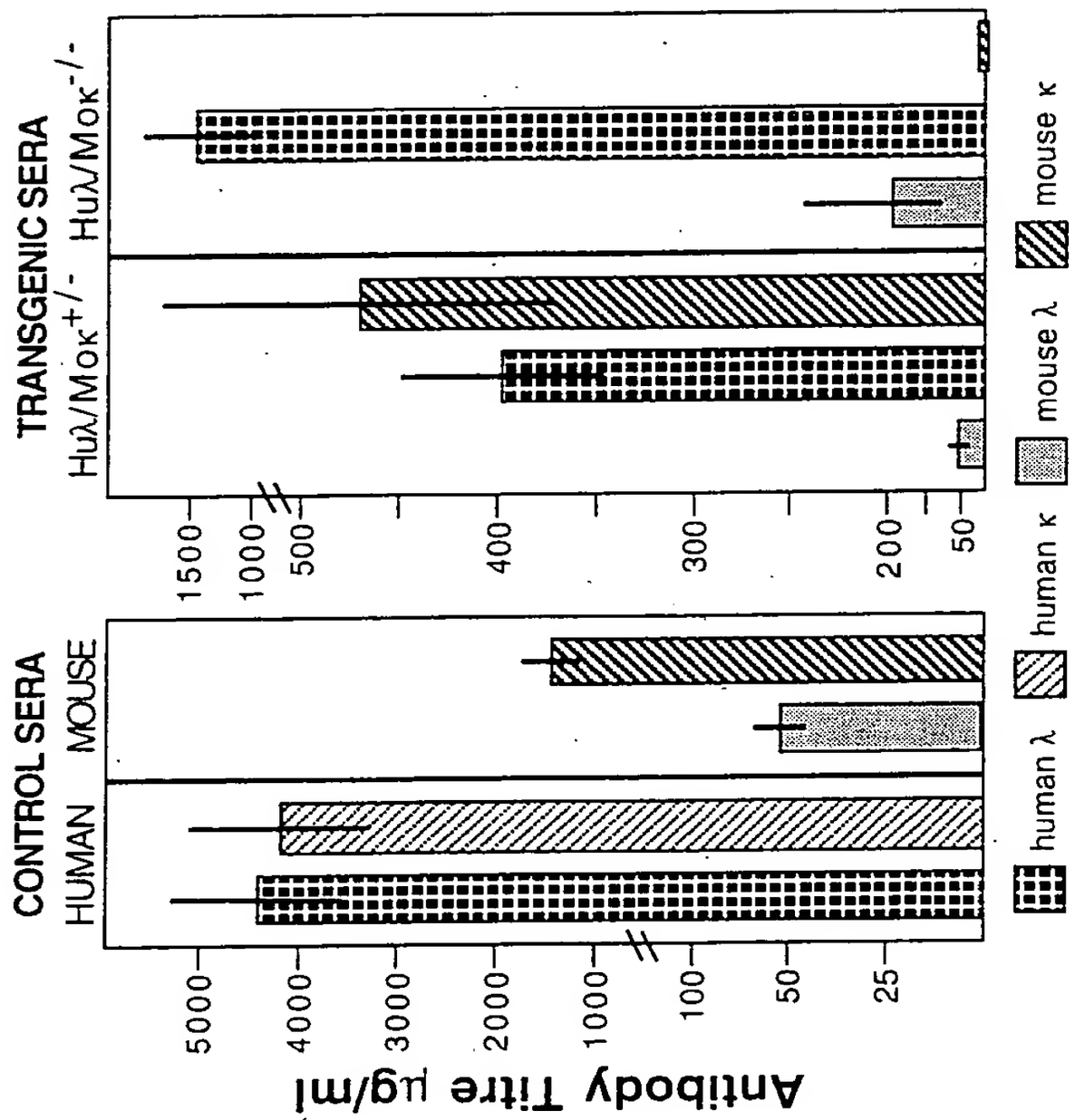
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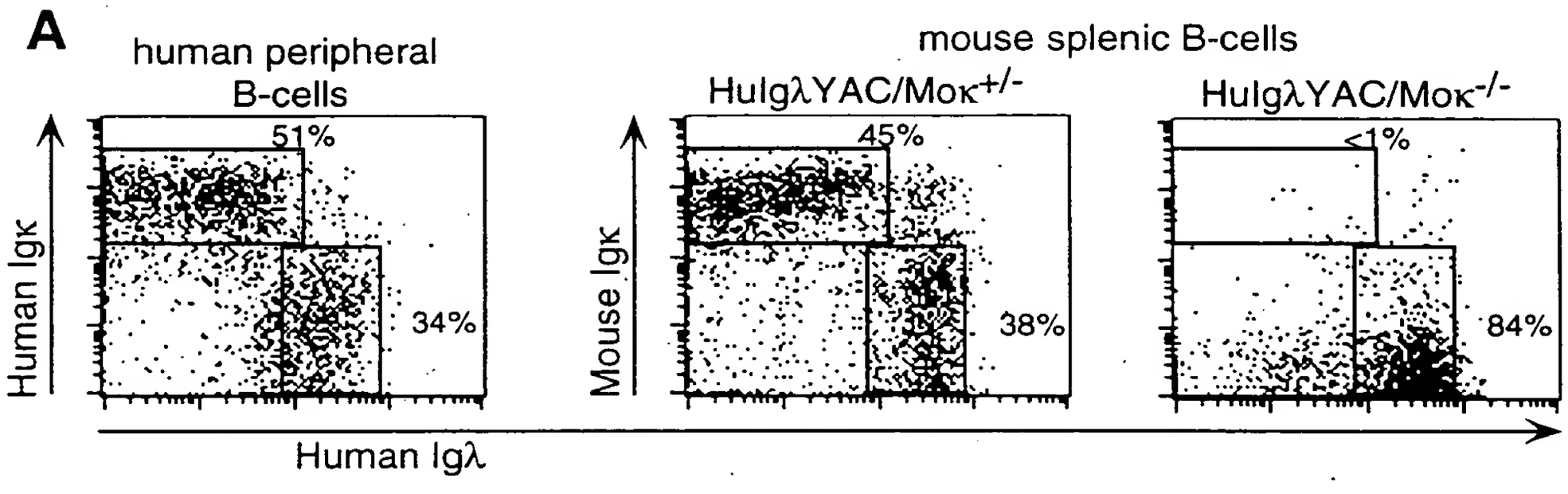

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                                     ____________CDR1________________
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14/9 VλO7 ... .A. ... ... ..T ... ... ... ... ... G.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A.T ... A.. ... ... ... .A. .G. ... ... ... ... ... ... ... ... ... ... ... ... ... ...>

                  210             225             240             255         _______CDR3_______
                                                                                    270         285
3-1       TCT GGG AAC ACA GCC ACT CTG ACC ATC AGC GGG ACC CAG GCT ATG GAT GAG GCT GAC TAT TAC TGT CAG GCG TGG GAC AGC AGC ACT GCA      T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA  Jλ3
14/9 VλO7 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... ... C.. ... ...       TGG GTA TTC GGC GGA GGG ACC TAC CTG ACC GTC CTG

                                     ____________CDR1________________
            60             75              90              105             120             135          ______CDR2_________ 165           180             195
                                                                                                         150
3-1       GCC AGC ATC ACC TGC TCT GGA GAT AAA TTG GGG GAT AAA TAT GCT TGC TGG TAT CAG CAG AAG CCA GGC CAG TCC CCT GTG CTG GTC ATC TAT CAA GAT AGC AAG CGG CCC TCA GGG ATC CCT GAG CGA TTC TCT GGC TCC AAC
14/9 VλO3 ... ... ... ... ... ..G A.. ... ... ... ... .A .C. ... .T. .C. ... ... .G. ... ... ... ... ... ... ... ... C.. ... ... ... .C. ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ...>

                  210             225             240             255         _______CDR3_______
                                                                                    270         285
3-1       TCT GGG AAC ACA GCC ACT CTG ACC ATC AGC GGG ACC CAG GCT ATG GAT GAG GCT GAC TAT TAC TGT CAG GCG TGG GAC AGC AGC ACT GCA      T GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA  Jλ2
14/9 VλO3 ... ... ... ... ... G.. ... ... ... .C. ... ... ... T.. ... ... ... ... ... ... ... ... ... ... ... ... GC. ... .        TG GTT TTC GGC GGA GGG ACC AAA CTG ACC ATC CTA

                                     ____________CDR1________________
            60             75              90              105             120             135          ______CDR2_________ 165           180             195
                                                                                                         150
3-10      GCC AGG ATC ACC TGC TCT GGA GAT GCA TTG CCA AAA AAA TAT GCT TAT TGG TAC CAG CAG AAG TCA GGC CAG GCC CCT GTG CTG GTC ATC TAT GAG GAC AGC AAA CGA CCC TCC GGG ATC CCT GAG AGA TTC TCT GGC TCC AGC
PP 05     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ... A.. ... ... ... ... ...>

                  210             225             240             255         _______CDR3______________
                                                                                    270         285
3-10      TCA GGG ACA ATG GCC ACC TTG ACT ATC AGT GGG GCC CAG GTG GAG GAT GAA GCT GAC TAC TAC TGT TAC TCA ACA GAC AGC AGT GGT AAT CAT AG      T TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA  Jλ3
PP 05     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ... ... ... ... ... A.. .C. ... ..       G GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA

                                     ____________CDR1______________________
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2-14      ATC ACC ATC TCC TGC ACT GGA ACC AGC AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAG CAC CCA GGC AAA GCC CCC AAA CTC ATG ATT TAT GAG GTC AGT AAT CGG CCC TCA GGG GTT TCT AAT CGC TTC TCT
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PP 14     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G.. ... ... ... ... ... ... ... ...        TGG GTG TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA

                                     ____________CDR1________________
            60             75              90              105             120             135          ______CDR2_________ 165           180             195
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PP 02     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ... ... ... ... .C. ... ..G ... ... ... ... ... ... ... ... ... ... ... ... ... ...>

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                                                                                    270         285
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PP 02     ... ... ... ... ... ... ... ... ... ... ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G.A ...          GTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA

                                     ____________CDR1______________________
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SORT 04   ... ... ... ... ... ... ... ... ... ... G.. ... ... ... ... ... T.. ... ... ... ... ... .TA ... ... ... .T. ... ... ... ... ... ... .A. .A. .T. ... ... ... ... ... ... ... ... ... ..T ...>

                  210             225             240             255             270   _______CDR3_______
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SORT 04   .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A.. C.. GA. .   GG   TGG GTG TTC GGC GGA GGG ACC AAC CTG ACC GTC CTA
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